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Acoustic startle reflex and pre-pulse inhibition in tinnitus patients

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Abstract

Gap induced pre-pulse inhibition (Gap-PPI) of acoustic startle reflex has been used as a measurement of tinnitus in animal models. However, whether this test is sensitive to detect tinnitus in humans is still unclear. Based on the testing procedure used in animal studies, a human subject testing method was formulated and conducted to investigate if a similar result could be found in tinnitus patients. Audiologic and tinnitus assessments and acoustic startle reflex measurements were performed on seven tinnitus subjects and nine age matched subjects without tinnitus. There was no significant difference found between the control and tinnitus group on the Gap-PPI across the frequencies evaluated. The amplitude of the startle response in the tinnitus group with normal hearing thresholds was significantly higher than the control group and those with tinnitus and hearing loss. This preliminary result suggests that hyperexcitability in the central auditory system may be involved in tinnitus. There was no correlation between hearing thresholds and the increased amplitude of startle response.

Keywords: Tinnitus; Startle reflex; Prepulse inhibition; Hearing loss

1. Introduction

Tinnitus is a phantom auditory perception often described as a ringing, buzzing, chirping or whooshing noise in the ear despite the absence of an external sound source (Jastreboff, 1990). This phantom sound can be constant or intermittent and range in severity from being barely noticeable to causing extreme distress, even suicide (Saunders, 2007). The variability in how tinnitus is perceived lends itself to the different thoughts of what the causes of tinnitus are, and where this auditory stimulation originates. The current focus of research is on spontaneous neuron discharges (Norena et al., 2003), although the peripheral auditory system and neuroplasticity in the regions of the brainstem and auditory cortex (Saunders, 2007) or any combination thereof have also been hypothesized as possible etiologies. The exact cause of tinnitus is still not clear.

It is believed that up to 10% of the general population perceives tinnitus. Veterans receive service related compensation for their tinnitus (Saunders and Griest, 2009). However, there are few objective, concrete measurements for the manifestation of tinnitus (Lockwood et al., 1998). The large numbers estimated of those who suffer from tinnitus and the amount of compensation for tinnitus are based solely on subjective measures which can lead to false positives and other unwanted situations. The need for an objective test for tinnitus is obvious.

The acoustic startle reflex (ASR) is a sudden muscular movement in response to a loud unexpected sound (Arnfred et al., 2004) which has been used to detect tinnitus in animal models (Engineer et al., 2011; Turner et al., 2006; Yang et al., 2007). The ASR pathway begins in the peripheral auditory system and moves up the brainstem to the reticular

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formation which then descends back to the spinal motor neurons leading to subsequent muscle movement. This pathway is thought to be the reason for the rapid reaction to the stimulus, as short as 8 ms in animals and ranging in speed from 10 ms to 150 ms in humans. There are a number of factors which may affect the ASR (Faraday and Grunberg, 2000). One such factor that can affect the ASR is a change in stimulus prior to the startling sound which leads to inhibition of the ASR response (Arnfred et al., 2004). This change in stimulus is known as a pre-pulse and the resulting inhibition is called the pre-pulse inhibition (PPI). Aurally this pre-pulse can be presented as a noise burst pre-pulse or in contrast by a silent break in continuous noise known as a gap pre-pulse. Recently research has begun in testing rats with tinnitus and the effects of tinnitus on PPI and the acoustic startle reflex (Chen et al., 2013; Turner et al., 2006; Yang et al., 2007). Research by Yang et al. showed statistically significant results using gap pre-pulse at the frequency in which tinnitus occurs in rats treated with salicylate (Sun et al., 2009; Yang et al., 2007). Turner et al. has shown that rats with tonal tinnitus exhibited less pre-pulse inhibition utilizing a gap pre-pulse than controls when the background noise was composed of the frequency similar to the rats' tinnitus (Turner et al., 2006), further lending to the idea that poor gap detection in tonal background noise is related to tinnitus.

The purpose of this study is to gauge the possibility of an objective means of tinnitus testing utilizing the gap induced pre-pulse inhibition of ASR in narrowband background noise. Using the knowledge gained through previous research on rats, an objective means of tinnitus testing is seen as a possibility.

2. Methods

2.1. Subjects

Two groups of subjects (male and female, 20–55 years old) were used in this project. The control group consisted of individuals without complaints of tinnitus and with hearing within normal limits (hearing thresholds are above 20 dB HL). The test group consisted of individuals suffering from tinnitus. As it is often found that persons suffering from tinnitus have a hearing loss, hearing losses through the moderate range were accommodated for and included in this group, though hearing within normal limits was considered to be optimal.

2.2. Audiology tests

The subjects first received a brief hearing screening. Otoscopy was done first, followed by a screening tympanogram of both ears to check for normal ear canal volume, pressure and compliance. A screening acoustic reflex at 1000 Hz was included. Air conduction threshold checks were conducted utilizing a GSI 61 audiometer and insert ear phones (ER-3A). Pure tone testing was conducted at 0.25, 0.5, 1, 2, 4, and 8 kHz for subjects with no complaints of tinnitus. Subjects with tinnitus were tested at the additional frequencies of 3 and 6 kHz, using a pulsed tone. Tinnitus pitch and loudness matching was performed and a brief questionnaire about their tinnitus was also given.

2.3. Acoustic startle reflex using Gap-PPI

Subjects were instructed to keep their eyes open, to remain awake and were informed about the sounds they would be hearing via the headphones. Lights in the testing booth were turned off to assist ;in reducing unnecessary muscle activity. Electrode site placement occurred at the locations immediately next to the outer corner of the subjects right eye (ground electrode) and below their right eyes, just above the inferior margin of the orbital socket (active electrode) (Hawk and Kowmas, 2003). The signal was amplified by a TDT preamplifier (RA16PA and RA16LI). The noise, both background and startle sound was calibrated (824, Larson Davis) with a ½ inch microphone (2540). The background noise was a narrowband noise with a 100 Hz bandwidth presented at 38–40 dB SPL centered at frequency of patient's tinnitus. The startle noise was a broadband signal at 100 dB SPL.

Startle reflex testing was controlled via the appropriate software. The inter-trial interval was random with a variation of 20–30 s. The gap duration was 100 ms and the duration of startle stimulus was 50 ms (rise/fall time 1 ms). Gap and no gap trials of the various frequencies tested were presented at random with a total of ten trials at each frequency (0.5–8 kHz). For subjects with a hearing loss, the startle schedule was modified to the fullest extent possible to accommodate for the increased threshold.

2.4. Data Analysis

All the data was organized and analyzed through Microsoft Excel and GraphPad Prism software. The gap-PPI was calculated based on the formula: (EBAng-EBAg)/EBAng x 100% (EBAng is the amplitude of the eye-blink measured in the presence of continuous noise and EBAg is the amplitude in the presence of gap).

3. Results

3.1. Audiologic tests

All 16 test subjects received an audiologic assessment prior to the startle reflex testing. Patients with external or middle ear dysfunction were excluded, with the exception of one subject in the tinnitus group who had a long standing tympanic membrane perforation. Comparison of the hearing thresholds between the control and tinnitus groups revealed a significant difference between the thresholds of the two groups (Fig. 1).

A screening acoustic reflex at 1 kHz was performed on all subjects as part of the audiologic assessment. In comparison of the control group to the tinnitus group it was found that the acoustic reflex was often absent in subjects of the tinnitus group, while the reflex was present in the subjects of the control group.

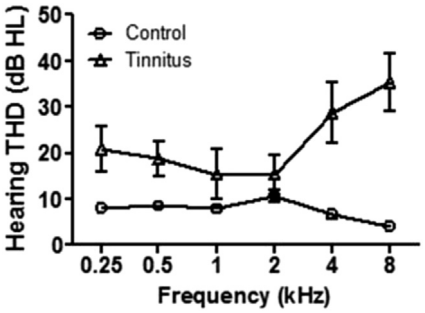

Fig. 1. Pure tone threshold in the control group (n = 9) and tinnitus group (n = 6). There was a significant difference between two groups (Two-way ANOVA, $p < 0.05$).

3.2. Gap-PPI and acoustic startle amplitude

All the subjects ASR responses were averaged within each group across frequencies. The average gap-PPI per frequency between groups was shown in Fig. 2. The control group and the tinnitus group show similar levels of inhibition. A two-way ANOVA test was performed to see if there was a difference between groups. Fig. 2 depicts that both the control and tinnitus group's data overlap and there was no significant difference in inhibition of the ASR found between the two groups.

For the tinnitus group individual subject gap-PPI responses were compared to their subjective perception of tinnitus (Fig. 3). The red rectangles in Fig. 3 depict where the subject perceived their tinnitus. The edge or a drastic change in the PPI in relation to pitch of perceived tinnitus was studied. For patient A017 and A011, the pitch of tinnitus was found on the edge of Gap-PPI (Fig. 3A, B). However, for patient E012 and J013, there was no correlation between tinnitus pitch and the edge of the gap-PPI (Fig. 3C, D).

Amplitude of the acoustic startle reflex of the tinnitus group was compared to the amplitude of the control group. There was no significant difference between the control and the tinnitus groups (Fig. 4A, Two-way ANOVA, $P > 0.05$). Analysis was taken a step further and the tinnitus group was broken down into those with and without hearing loss. It was found that the acoustic startle amplitude in tinnitus subjects

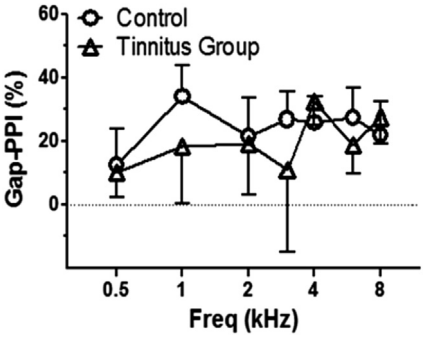

Fig. 2. Averaged gap induced pre-pulse inhibition in the control group (n = 9) and the tinnitus group (n = 6). There was no significant difference between these two groups.

without hearing loss (<20 dB HL in all frequencies, Tinnitus-No-HL, n = 4) was significantly higher than the control and tinnitus subjects with hearing loss (>20 dB HL, Tinnitus-HL, n = 3) (two-way ANOVA, $F(2, 63) = 7.85$, $P < 0.001$). These results suggest that hyperexcitability in the central auditory system may be involved in tinnitus.

4. Discussion

In this study, we measured acoustic startle response using eye-blink amplitude in human subjects. We found a reduction of gap-PPI in tinnitus subjects. However, we did not find a significant difference between the control group and the tinnitus group. This may be affected by the diverse causes and degrees of tinnitus. For example, in our tinnitus subjects, tinnitus was perceived differently – ringing, buzzing, chirping or whooshing noise. Knowing this, the ability to gather a group of tinnitus patients having the same tinnitus – matching loudness and pitch – and having a similar audiologic threshold is nearly impossible. This lack of control will be inherent in research performed utilizing this method. Another reason which leads to a large standard error is the small sample size. A larger sample size would increase the power and strength of the testing and perhaps lead to much different research results.

Another consideration involving the range of tinnitus perception is the ability to accurately recreate an individual's tinnitus perception. In order to generate specifically a stimulus which would mimic a patient's tinnitus a multitude of hardware aperture would be required, if the ability to directly mimic an individual's tinnitus is even possible. For our research we used 100 Hz band pass filtered narrowband noise centered at the target frequency in the 0.5–8 kHz bandwidth. This continuous background noise may not have effectively mimicked tinnitus. Thus the "gap" of the continuous narrowband noise may not have been perfectly filled in during this study. The considerations of a subjects' tinnitus may help explain why we were unable to find the same results as the animal studies.

Attention has also been shown to modulate a person's ASR. Filion et al. has shown that paying attention leads to greater inhibition as opposed to inattention (Filion et al., 1993). During startle reflex testing subjects were not expressly told to actively listen for the gaps in the background noise. Adding in instruction for the subject to actively listen for the gaps in background noise could lead to better results. This would hypothetically lead to a greater decrease in startle response amplitude when the pre-pulse condition is detected.

During our research fifteen subjects, or thirty ears, had hearing within normal limits. We found that eight of fourteen ears, or 57%, of the tinnitus group did not have an acoustic reflex. For the control group only three of eighteen ears, or 17%, did not have an acoustic reflex. There is clearly a significant difference between the control group and the tinnitus group's acoustic reflex response. In a separate finding the acoustic startle amplitude in tinnitus subjects who have normal hearing thresholds was significantly larger than control

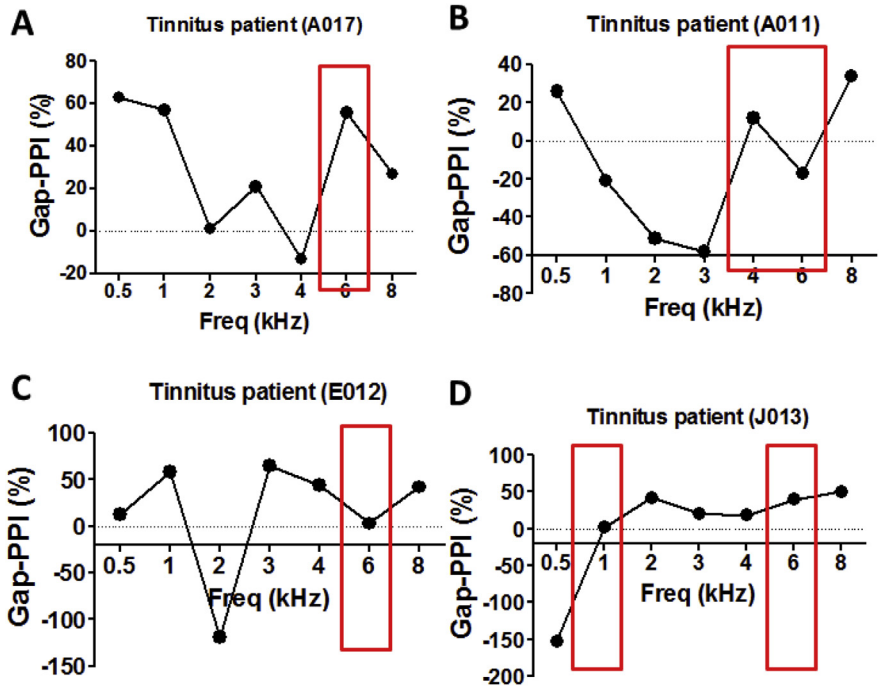

Fig. 3. Gap-induced prepulse inhibition (Gap-PPI) in individual subjects in the tinnitus group. The red rectangle depicts the subjects perceived tinnitus pitch. (A−B) Tinnitus pitch was located at the frequency of the notch of Gap-PPI. (C−D) Tinnitus pitch was not correlated with notch of Gap-PPI.

subjects. This result is consistent with a recent study that showed tinnitus patients had enhanced acoustic startle amplitude than subjects without tinnitus (Hebert et al., 2013). Their results may be interpreted that tinnitus patients often show less tolerance to loud sound, known as hyperacusis (Hiller and Goebel, 2006; Juris et al., 2013). Although we did not know the cause of this difference, it does bear consideration for further tinnitus research.

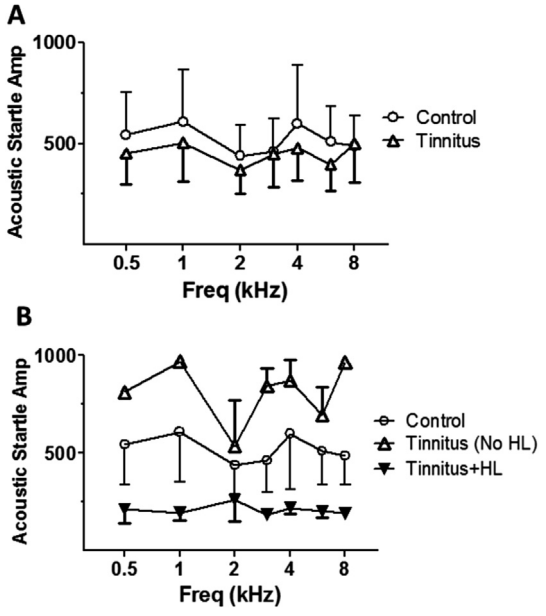

Fig. 4. The acoustic startle amplitude of the tinnitus group and the control group. (A) There was no significant difference between the control and the tinnitus groups (two-way ANOVA, $P > 0.05$). (B) The acoustic startle amplitude in tinnitus subjects without hearing loss (<20 dB HL in all frequencies, Tinnitus-No-HL, $n = 4$) was significantly higher than the control and tinnitus subject with hearing loss (>20 dB HL, Tinnitus-HL, $n = 3$) (two-way ANOVA, $F(2, 63) = 7.85$, $P < 0.001$).

In summary, the purpose of this research was to evaluate an objective means of tinnitus testing by measuring the changes in inhibition of the acoustic startle reflex, by presenting brief silent gaps in continuous noise at frequencies near the pitch of the subjects' perceived tinnitus. A significant difference was not found between the control and tinnitus groups on the inhibition of the acoustic startle reflex in response to the gap pre-pulse. However, the acoustic startle amplitude of those with normal hearing in the tinnitus group was significantly larger than control subjects. Further research may be conducted in regards to an expanded sample size and improved tinnitus matching stimuli for the background noise of the acoustic startle reflex. Continued investigation of the acoustic startle reflex testing in human subjects with tinnitus will help guide the possibility of this testing being used for an objective means of tinnitus evaluation.

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